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Characterization for Multipurpose Exploitations of Genetic Resources from the Germplasm Collection of Pasture Species Owned by the CNR-ISPAAM in Sassari, Italy

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Summary: This paper reports the results of genetic resources characterization of some pasture species from the germplasm collection held at ISPAAM-CNR in Sassari, Sardinia, Italy. According to the peculiarities of each species, some of the uses suggested by the experimental results were phytoremediation, wildfire prevention, biomass production for bioenergy, forage production and multiple uses, bioactive compounds for health care of domestic animals.
Key words: bioactive compounds, forages, Mediterranean germplasm, non-conventional uses

Introduction

The ISPAAM u.o.s. of Sassari (formerly Center for Studies on Mediterranean Pastures) was established by the Italian National Research Council in 1984. During the last twenty five years, a main research topic has been the identification and evaluation of genetic resources of forage interest for their introduction into cropping systems for production purposes and for multiple uses, including land recovery and environmental conservation. In such a frame, local germplasm of several species was collected and evaluated for biological and morphological characters useful for forage or multiple uses in the Mediterranean areas. The Institute holds a collection of several herbaceous species, belonging to the more traditional botanic families *Leguminosae* and *Gramineae*, and also more unusual species belonging to the *Compositae* and *Geraniaceae*, all isolated from the local flora and characterized for biological and morphological traits useful for fodder and multipurpose uses in the Mediterranean environments. A brief description of the collection and the results of some experimental trials aimed at the characterization of accessions of a few species are reported here. Attention is focused on the germplasm characterization for phytoremediation, wildfire prevention, biomass production for bioenergy, forage production and multiple uses, bioactive compounds for health care of domestic animals.

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The Germplasm Collection of Pasture Species

Sixty two species, from the botanic families *Leguminosae*, *Gramineae*, *Compositae*, *Geraniaceae* and *Plantaginaceae*, are present in the germplasm collection owned by the CNR-ISPAAM u.o.s. of Sassari. The collection includes twenty-five genera, with the genus *Trifolium* and *Medicago* being the most represented with 16 and 15 species respectively. All species in the collection are spontaneous in the Mediterranean flora and are interesting for their potential in multipurpose uses. The germplasm collection includes 547 accessions listed in table 1. Almost all accessions were collected in the regional territory of the island of Sardinia. Annual species are more widespread than perennials in Sardinian pasturelands due to their higher diffusion in the regional territory. This is mainly due to the shallow soil layers that hinder the persistence of perennials during summer drought, to the high grazing pressure that does not allow seed formation of perennials and their natural regeneration on pasturelands. Nevertheless, where perennials are present, they are well adapted to the Mediterranean climate, due to their summer dormancy and good winter growth, features that make them valuable resources for breeding new varieties of perennial

species suitable for Mediterranean environments. All accessions of the collection represent an important genetic reservoir, also considering the environmental issues deriving from the overgrazing, wildfires, human activities impacts on environment and land desertion occurring in marginal Mediterranean areas.

Genetic Resources for Phytoremediation Purposes

The project "Development of symbiotic systems between pasture legumes and soil microorganisms to restore soil fertility in arid and polluted areas", selected in the frame of the third

Table 1. Species in the germplasm collection of the CNR-ISPAAM u.o.s. Sassari, Italy with indications of species already characterized for forage (f), environment (e) or bioenergy (b) purposes.
Tabela 1. Biljne vrste u kolekciji germplazme CNR-ISPAAM, Sasari, Italija sa indikacijom vrsta čija je upotreba karakterizovana za krmu (F), životnu sredinu (e) i bioenergiju (b)

Species Vrste	N. of accessions Br. populacija	Species Vrste	N. of accessions Br. populacija
Aegilops sp. (e)	1	*Medicago praecox*	1
Anthyllis sp. (e)	1	*Medicago rigidula*	2
Astragalus boeticus (e)	2	*Medicago rugosa*	4
Astragalus hamosus (e)	5	*Medicago sativa* (f)	10
Biserrula pelecinus (f)	4	*Medicago scutellata*	1
Cichorium intybus (f, e)	7	*Medicago truncatula*	13
Chrisanthemum coronarium (f)	3	*Melilotus* sp.	2
Cynodon dactylon (e)	1	*Melilotus sulcata*	1
Cynosurus cristatum	1	*Melilotus messanensis*	1
Dactylis glomerata (f)	41	*Ornithopus compressus* (f,e)	2
Dactylis hispanica	1	*Phalaris aquatica*	25
Erodium moschatum (f, e)	1	*Plantago lanceolata*	4
Festuca arundinacea (f, e)	10	*Sylibum marianum* (f, b)	5
Hedysarum coronarium (f, e)	15	*Scorpiurus muricatus* (f,e)	3
Hippocrepis multisiliquosa (e)	1	*Trifolium campestre*	1
Hymenocarpos sp.	1	*Trifolium cherleri*	2
Lathyrus sp.	3	*Trifolium angustifolium*	1
Lolium multiflorum (f)	2	*Trifolium campestre*	2
Lolium perenne (f)	21	*Trifolium cherleri*	1
Lolium rigidum (f, e)	77	*Trifolium glomeratum*	1
Lotus cytisoides (f, e)	5	*Trifolium ligusticum*	2
Lotus edulis (f, e)	4	*Trifolium nigrescens* (f,e)	41
Lotus ornithopodioides (f, e)	8	*Trifolium pratense* (f)	7
Medicago aculeata	1	*Trifolium repens* (f)	14
Medicago arabica	14	*Trifolium resupinatum*	1
Medicago ciliaris	1	*Trifolium scabrum*	3
Medicago litoralis	1	*Trifolium spumosum*	2
Medicago marina	1	*Trifolium squarrosum*	2
Medicago minima	7	*Trifolium subterraneum* (f,e)	60
Medicago murex	29	*Trifolium tomentosum*	2
Medicago orbicularis	4	TOTAL	547
Medicago polymorpha (f, e)	58		

executive program of scientific and technologic cooperation between Italy and Russia, has been developed in cooperation with scientists from ARRIAM (All Russia Research Institute for Agricultural Microbiology). The project is aimed at identifying plant species tolerant to heavy metals (HMs) and efficient plant-bacteria associations to promote plant growth and root nodulation activity under stress conditions, suitable for phytoremediation purposes. The characterization of different rhizobial strains, symbionts of several Mediterranean pasture legumes species, the identification of some plant-microbe interactions to develop successful techniques for phytoremediation, the selection of rhizobacterial strains particularly efficient in heavy metal polluted soils are among the scientific results of the cooperation (Safronova et al. 2004, Belimov et al. 2005). We identified and collected local populations of legume and non legume species that are able to colonize polluted soils of Sulcis mining region (SW-Sardinia) where concentration of Cd, Zn and Pb can respectively reach about 36, 5900, 2800 mg kg^{-1} in mine waste areas. We performed hydroponics and pot soil experiments to compare tolerance to HMs of several pasture legume populations collected from polluted and non-polluted sites of Sardinia, and then we estimated the response of the selected plants to inoculation with PGPR containing ACC deaminase and with nodule bacteria, in the presence of elevated HM concentrations. The aim of designed experiments was to identify HM tolerant legume-microbe systems for phytoremediation of mine wastes (Safronova et al. 2010a). Significant interspecific differences in the growth, HM relations and response to inoculation with microorganisms were found. Efficient and metal tolerant plant-microbe associations were detected. Synergistic and additive effects on plant growth and nutrition were observed in *Lotus* spp. grown in HM-polluted soil after combined inoculations with nodule bacteria *Mesorhizobium loti* and PGPR *Variovorax paradoxus*. It is important to collect germplasm from contaminated areas and bring it into cultivation, for establishing germplasm collection and facilities useful for phytoremediation purposes, and for research aimed at selecting fast growing and tolerant genotypes able to colonise polluted areas. A detailed review on the benefits and potential problems in the use of legume-microbe symbioses for phytoremediation of heavy metal polluted soils has recently been completed in the frame of the collaboration with the Institute ARRIAM of Saint Petersburg (Safronova et al. 2010b).

Genetic Resources for the Wildfire Prevention

According to the experiences from Sardinia by Franca et al. (2003), firebreaks management by the oversowing of well-adapted pasture species and a correct grazing pressure can allow the constitution of a nine-month green covered firebreak, rotationally grazed by animals and during summer a dry and low–risk firebreak, owing to the control of the fuel biomass made by the animals. Such method represents a valid multi-use system that protects landscape and soil, preserves biodiversity and decreases management costs, compared to the traditional management of firebreaks tillage. Experiences on such a topic by Franca et al. (2003) indicated that: 1) low input management (no-tillage plus low seed rate) with sowing of local ecotypes and commercial varieties of annual self-reseeding legumes (*Trifolium spp* and *Medicago polymorpha* L.) and grasses (*Lolium rigidum* Gaudin) in pure stand or mixture were satisfactory. It allowed the establishment of a complete and persistent herbage cover, to be grazed for reducing fuel accumulation; 2) the management of grazing pressure and/or cutting regimes resulted a parameter as determinant as the green cover establishment; 3) the best management strategy seemed to be a combination of herbage grazing and/or residual cutting. To achieve a suitable forage production, soil protection and fire prevention, it seems crucial to choose adequate plant materials. According to Franca et al. (2003), local flora represents a valuable source of ecotypes to be efficiently utilised in low input firebreaks management.

Genetic Resources of Non-Conventional Species for Forage and Multiple Uses

Encouraging results were obtained by Sulas et al. (2007a, 2007b, 2007c) in the experiences aimed at the characterization for introduction into cropping of local natural populations of *Erodium moschatum* (L.) L'Herit., *Cichorium intybus* (L.) and *Chrysanthemum coronarium* (L.). *E. moschatum* showed appreciable nutritional characteristics for ruminants, mainly in autumn, and growth habitus and roots development apt to soil preservation on slopes. According to Sulas et al. (2007a), the *E. moschatum* first cut plus its regrowth yielded about 2 t of DM ha^{-1}. The *E. moschatum* herbage dry matter percentage was about 10%; crude protein (CP) ranged from 15% to 20% while NDF and ADF contents were moderate. These results indicate that the forage

could have a good digestibility. Moreover, the total polyphenols content was on average 5.5% DM and it was similar to that of Mediterranean forage legumes. *C. intybus,* positively assessed for forage production and quality, can be considered as a valid alternative or, anyway, a complementary species to traditional grass and legume forages. According to Sulas (2007b), on the average of the triennium of trial, DMY per year of a local population of *C. intybus* reached 7.2 t ha^{-1} and 6.8 t ha^{-1} under undisturbed and cut plots, respectively. This suggests that a frequent utilization is profitable for the annual Sardinian population of forage chicory, whose yields from contrasting treatments were surprisingly similar. Moreover, *C. intybus* can also have a role in environment preservation to protect soil and for landscape embellishment. *C. coronarium,* considered in the past only as a weed, showed remarkable forage productions and good silage quality; for such species it has also been successfully set up the mechanical harvesting of seed, a very important step for future introduction into cropping (Sulas et al. 2007c).

Genetic Resources for Bioenergy from Biomass Production

A seed collection of the Sardinian populations of milk thistle (*Silybum marianum* Gaertner) was made and plots of such germplasm were established in 2006 to evaluate the performance of the species in terms of biomass production under low input conditions. Sulas et al. (2008) report the results of such experience in terms of dry matter accumulation, yield and biomass partitioning into its components (leaves, stems and heads), heating value and energy balance of the crop. According to Sulas et al. (2008), although the species is reputed a dangerous weed in cropping areas, the rusticity and the high growth rate of *S. marianum* make such species very interesting for bioenergy purposes under rainfed Mediterranean conditions, particularly in those areas where traditional cropping surfaces have recently decreased. The same authors report that about 20 t ha^{-1} of dry biomass were produced in June and remark that a low level of inputs were applied, making the species interesting for biomass production. They indicate that the high heating value (HHV) was on average 14.8 MJ kg^{-1} of DM, and report a total energy requirement for cultivation of the species of 8.0 GJ ha^{-1}, 55% of which is represented by indirect energy. Considering an average calorific value of the whole dry biomass of 14.8 GJ t^{-1}, the gross energy output was 301.3 GJ ha^{-1}, with a net energy gain of 293.3 GJ ha^{-1} (Sulas et al. 2008). The authors remark that such a value, compared to the output to input energy ratios of other crops such as *Arundo donax* and *Sorghum bicolor*, indicate that *S. marianum* has a better efficiency in energy conversion as well as energy productivity (2.5 GJ per each ton of biomass produced).

Genetic Resources for Traditional Health Care of Domestic Animals

A study about the diffusion of traditional herbal remedies for health care of domestic animals in Sardinia was made by Bullitta et al. (2007) by means of expeditions in the regional territory, interviews with animal breeders and filling questionnaires. The monitoring of knowledge related to plant species utilised in the past for traditional veterinary practices favoured the recovery of ancient local traditions related to veterinary ethnobotany and also the identification of potential plant resources useful for developing new natural products for animal phytotherapy and as feed additives, to improve animal performances and productions for organic animal farming. According to the information collected, 85 remedies of plant origin were identified. The number of species utilised for the preparations were 41, 9 of which cultivated and 32 from the wild flora. It was found that 19 out of the 41 species are still occasionally used for preparing herbal remedies for domestic animals. Thirteen species were also used to prepare herbal remedies for humans. The plant families mostly represented were *Liliaceae* and *Leguminosae* with four species each and *Rosaceae* and *Umbelliferae* with three species each. *Vitis vinifera*, *Olea europaea* L. var. *europaea*, *Pistacia lentiscus* L., *Quercus suber* L., *Malva sylvestris* and *Parietaria officinalis* were the species mostly utilized to prepare herbal remedies for domestic animals. The following step in the research was determination of antioxidant properties and correlations between phenolic content and antioxidant properties in 24 plant species of traditional ethnoveterinary use in the Mediterranean area (Piluzza & Bullitta 2010a)

Another topic of research related to the animal well-being is the study of phenolic concentration dynamics in some pasture species and the implications for animal husbandry (Bullitta 2005). Although secondary metabolites have often been studied considering their primary role as plant defensive and their effect on consumers detrimental, their effects are usually dose dependent and it is important to consider their content in all phenological phases and in different

plant organs. In such a frame, a survey was made on the content of total phenolics (TotP), non-tannic phenolics (NTP) and proanthocyanidins (PA) in natural populations of pasture species at defined phenological phases and in different plant organs, and also on pathogen infected plants (Piluzza & Bullitta 2010b). The species included in the study were *Hedysarum coronarium* L., *H. spinosissimum, H. glomeratum* Dietrich, *Onobrychis viciifolia* Scop., *Lotus edulis* L., *L. ornithopodioides* L., and *L. cytisoides* L., collected from local natural populations in semi-arid pastureland areas of Sardinia, Italy. Variation in TotP, NTP and PA content was observed in all species, phenological phases and plant organs. Variations in TotP, NTP and PA content were observed either within and between species in all the phenological phases and plant organs that were examined**.** For instance**,** at the flower bud appearance phase, we found differences up to 35%, 78% and 31% respectively in TotP, NTP and PA concentrations among the leaf blades of eight natural populations of *H. coronarium* (Piluzza & Bullitta 2010b). Such finding could affect the nutritional value and the effects of PA on grazing animals. Moreover, it should be considered that the localization of PA in defined plant tissues and changes in PA concentration associated with plant maturity will affect palatability and ultimately the intake of forage consumed by animals. PA concentrations were always in the range considered beneficial for animals, not exceeding 60 mg delphinidin equivalent g^{-1} DM.

Conclusions

The few examples reported on the characterization for production and multiple uses of genetic resources from Sardinian natural pasturelands confirm the idea that native species from Mediterranean pasturelands can be suitable materials for the selection of new varieties. Such genetic resources seem promising materials to accomplish the current requirements of multifunctional agriculture for production, energy, environment and public health needs.

References

Belimov A A, Hontzeas N, Safronova V I, Dodd I C, Demchinskaya S V, Piluzza G, Bullitta S, Davies W J, Glick B R (2005): Cadmium tolerant root growth-promoting bacteria of the rhizoplane of Indian mustard (*Brassica juncea* (L.) Czern). Soil Biol. Biochem 37: 241-250

Bullitta S (2005): Bioactive Compounds in Pasture species for phytotherapy and animal welfare. (issued from the Anfit-Mi-PAF Project). ISBN 88-901771-1-X, Sassari, Italy

Bullitta S, Piluzza G, Viegi L (2007): Plant resources used for traditional ethnoveterinary phytotherapy in Sardinia (Italy). Genet Res and Crop Evol 54 (7): 1447-1464

Franca A, Seddaiu G, Caredda S (2003): Two sylvopastoral approaches for the wildfires prevention in Sardinia. In: A. Franca (ed.) The future of the green Mediterranean. Euro-Mediterranean Conference. Arti Grafiche Pisano Cagliari

Piluzza G, Bullitta S (2010a): Correlations between phenolic content and antioxidant properties in twenty four plant species of traditional ethnoveterinary use in the Mediterranean area (submitted to Pharmaceutical Biology)

Piluzza G, Bullitta S (2010b): The dynamics of phenolic concentration in some pasture species and implications for animal husbandry. J. Sci. Food Agric. **(**Published Online: Apr 13 2010 9:14AM DOI: 10.1002/jsfa.3963)

Safronova V I, Piluzza G, Bullitta S, Belimov A A (2010): Use of legume-microbe symbioses for phytoremediation of heavy metal polluted soils: advantages and potential problems (in press Nova Publishers, New York)

Safronova V I, Piluzza G, Belimov A A, Bullitta S (2004): Phenotypic and genotypic analysis of rhizobia isolated from pasture legumes native of Sardinia and Asinara island. Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol. 85: 115-127

Safronova V I, Piluzza G, Belimov A A, Bullitta S (2010): Bacterial assistance in the growth, nutrition and heavy metal uptake of pasture legumes grown in polluted mine waste (submitted to International Journal of Phytoremediation)

Sulas L, Canu S, Piluzza G, Sassu M, Stangoni A P (2007a): Ecological and forage attributes of *Erodium moschatum* (L.) L'Herit. In: S. Bullitta (ed.), Plant Genetic Resources of Geographical and "other" Islands. (Conservation, evaluation and use for plant breeding), 159-160

Sulas L, Canu S, Muresu R, Piluzza G (2007b): A new annual forage cichory (*Cichorium intybus* L.) from Sardinia. In: S. Bullitta (ed.), Plant Genetic Resources of Geographical and "other" Islands. (Conservation, evaluation and use for plant breeding), 115-119

Sulas L, Saba P, Cesaroni C (2007c): Seed production and harvesting for the exploitation of *Chrysanthemum coronarium* L. germplasm. In: S. Bullitta (ed.), Plant Genetic Resources of Geographical and "other" Islands. (Conservation, evaluation and use for plant breeding), 157-158

Sulas L, Ventura A, Murgia L (2008): Phytomass production from *Sylibum marianum* for bioenergy. Options Méditerranéennes, Series A, 79: 487-490

Karakterizacija u cilju višenamenskog korišćenja genetičkih resursa iz zbirke pašnjačkih vrsta u institutu CNR-ISPAAM u Sasariju, Italija

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Izvod: Rezultati se odnose na karakterizaciju genetičkih resursa nekih pašnjačkih vrsta zbirke održavane u Institutu za stočarstvo u mediteranskim uslovima (ISPAAM-CNR) u Sasariju (Sardinija), Italija. U skladu sa osobinama svake vrste i rezultatima ispitivanja, mogući načini korišćenja uključuju fitoremedijaciju, prevenciju požara u divljini, proizvodnju bioenergije, proizvodnju krme i višestruke namene, kao i bioaktivne sastojke u veterinarske svrhe.
Ključne reči: bioaktivni sastojci, krmne biljke, mediteranska germplazma, nekonvencionalno korišćenje.